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Mesenchymal Stem Cell Therapy for Hematopoietic Stem Cell Transplantation with Poor Graft Function: A Single-Center, Randomized, Controlled, Open Clinical Trial

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Research Proposal Abstract

Title	Clinical Study on the Therapeutic Effect of Mesenchymal Stem Cells in the Treatment of Poor Graft Function after Allogeneic Hematopoietic Stem Cell Transplantation
Applicant	Second Affiliated Hospital of Army Medical University (formerly known as Third Military Medical University)
Primary Research Institution	Second Affiliated Hospital of Army Medical University (formerly known as Third Military Medical University)
Research enter	Second Affiliated Hospital of Army Medical University (formerly known as Third Military Medical University)
Research Objective	To explore the efficacy of umbilical cord-derived mesenchymal stem cells in the treatment of Poor Graft Function (PGF) after allogeneic hematopoietic stem cell transplantation.
Research Hypothesis	Umbilical cord-derived mesenchymal stem cells can treat impaired hematopoietic function after allogeneic hematopoietic stem cell transplantation.
Research Design	Single-center, randomized, controlled, open study.
Inclusion Criteria (Main)	1) Patients undergoing allogeneic hematopoietic stem cell transplantation. 2) Both genders, aged ⩾18 and ⩽60 years. 3) Karnofsky Performance Status (KPS) score >60, predicted survival >3 months. 4) No severe systemic organ dysfunction. 5) No contraindications for other hematopoietic stem cell transplantation. 6) Voluntary participation with informed consent.
Exclusion Criteria (Main)	1) Severe heart, kidney, or liver dysfunction. 2) Patients requiring treatment for other malignant tumors. 3) Clinical symptoms of brain dysfunction or severe psychiatric illness that impairs understanding or adherence to the research protocol. 4) Inability to complete the necessary treatment plan and follow-up observations. 5) Patients with severe acute allergic reactions. 6) Clinically uncontrolled active infection. 7) Patients currently participating in other clinical trials. 8) Other reasons deemed unsuitable for the clinical trial by the investigators.
Simple size	The ratio of cases between the experimental group and the control group is 1:1. Each group requires 31 cases, considering a possible dropout rate of 10%, resulting in a total of 34 cases per group.
Intervention	In addition to routine treatment for poor graft function, the experimental group will receive weekly infusion of umbilical cord-derived mesenchymal stem cells at a dose of 1×10^6/kg for four consecutive weeks.
Efficacy Indicators	Primary endpoint: Recovery of blood parameters in patients with poor graft function after treatment. Secondary endpoints: Infection rate, graft-versus-host disease,

	survival status (overall survival rate and disease-free survival rate within 1 year).
Safety Indicators	Incidence of other transplantation-related complications (infection, graft dysfunction, etc.) within 1 year after transplantation, acute adverse events, rejection reactions, and relapse rate within 1 year after transplantation.
Expected Timeline	September 2023 to December 2023: Design a single-center, randomized clinical research protocol and conduct its validation. January 2024 to December 2024: Initiate the clinical study, including ethics evaluation and international clinical research registration. Conduct a single-center, randomized clinical study on MSCs therapy for PGF, accumulate cases, and conduct mechanistic research. Prepare one research paper. January 2025 to October 2026: Continue the single-center, randomized clinical study on MSCs therapy for PGF, obtain midterm efficacy and safety data. Summarize the midterm data and prepare one research paper. November 2027 to September 2028: Complete the trial enrollment, continue follow-up, and summarize the clinical research data. Prepare one to two research papers.

1. Research Background

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for benign and malignant hematological disorders. It mainly involves high-dose chemotherapy to eliminate tumor cells, restore the recipient's hematopoiesis and immune function, and replace the recipient's hematopoietic stem cells (HSCs) with donor-derived HSCs to rebuild the donor's hematopoietic and immune function, thereby achieving the goal of curing the disease [1]. Successful engraftment of hematopoietic stem cells is characterized by the recovery of neutrophils, red blood cells, and platelets. Neutrophil engraftment is defined as a continuous presence of neutrophils exceeding 0.5×109/L for three consecutive days; platelet engraftment is defined as a continuous platelet count of at least 20×109/L for seven consecutive days without transfusion; and red blood cell engraftment is defined as a hemoglobin level of at least 70g/L without the need for blood transfusion [2-3]. Poor graft function (PGF) after transplantation is one of the main factors limiting the improvement of allo-HSCT efficacy. Therefore, it is of great clinical significance to explore the factors influencing successful engraftment of hematopoietic stem cells and new treatment strategies.

Mesenchymal stromal cells (MSCs) were first identified by Friedenstein in the bone marrow stroma in 1968 as a non-hematopoietic adult multipotent stem cell [4]. It has been found that MSCs can maintain and increase the specific colony-forming units of CD34+ hematopoietic stem cells [5], participate in the regulation of hematopoietic cell growth, and also have unique immunomodulatory characteristics. MSCs express moderate levels of human leukocyte antigen (HLA) class I molecules, do not express HLA class II molecules, FAS ligand, or co-stimulatory molecules such as B7-1, B7-2, and CD40L. They can inhibit the proliferation response of T cells in mixed lymphocyte cultures (MLCs) or mitogen stimulation. Animal experiments have shown that infusion of HLA-matched or "third-party" MSCs expanded in vitro can prolong the survival of allogeneic skin grafts [6], confirming the immunosuppressive effects of MSCs in vivo.

Risk factors for PGF after allo-HSCT include graft-versus-host disease (GVHD), the number of hematopoietic stem cells, anti-HLA antibodies (donor-specific antibodies) [7], previous alloimmune reactions, conditioning regimen intensity, cytomegalovirus (CMV) infection, graft source, low platelet count before conditioning, iron overload, etc. [8]. Currently, there is a lack of standardized treatment protocols for PGF after allo-HSCT, and treatment mainly focuses on addressing the underlying causes such as viral and infectious agents, hormones, etc. The intervention period is long, and the effective rate is less than 40%. Therefore, there is an urgent need to explore new and effective treatment methods. Multiple studies and preliminary research by our research team have indicated that mesenchymal stem cells (MSCs) can improve the bone marrow hematopoietic microenvironment, promote the proliferation and differentiation of hematopoietic stem cells, enhance hematopoietic function, and support hematopoiesis, as well as

directly or indirectly promote vascular regeneration in injured tissues and organs by secreting various cytokines. Existing research has confirmed a close association between the occurrence of PGF and endothelial damage in the bone marrow microenvironment, MSC damage, and imbalanced oxygen metabolism-induced HSC damage [9]. Therefore, exploring the treatment of PGF after transplantation from the perspective of improving the bone marrow hematopoietic microenvironment has certain feasibility. Currently, there is limited research on MSC treatment for PGF, and there are no reports on the use of systematic treatment protocols for PGF. Based on our previous research, we plan to adopt a safe and effective MSC treatment protocol for PGF and preliminarily elucidate its mechanism.

With the support of the National Key Research and Development Program Stem Cell Research and Organ Repair Major Project Subproject (2022YFA1103300, 2022YFA1103304), we have designed a single-center, prospective, randomized, controlled clinical study. We have also consulted renowned domestic experts in hematopoietic stem cell transplantation multiple times to develop the research protocol. For allo-HSCT patients, we will use 4th generation UC-MSCs, which have not undergone any genetic modification or gene editing. Based on conventional treatment for poor engraftment, umbilical cord blood-derived mesenchymal stem cells will be infused at a dose of 1×10^6/kg per week for four consecutive weeks. We aim to explore the efficacy of umbilicalcord blood-derived mesenchymal stem cells (UC-MSCs) in improving the engraftment of hematopoietic stem cells and the treatment of poor graft function (PGF) after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

The objective of this clinical study is to evaluate the safety and efficacy of UC-MSCs in improving the engraftment of hematopoietic stem cells and treating PGF after allo-HSCT. The study will be conducted as a single-center, prospective, randomized, controlled trial.

The study design involves two groups: the UC-MSC treatment group and the control group. Patients in the UC-MSC treatment group will receive UC-MSC infusions at a dose of 1×10^6/kg per week for four consecutive weeks in addition to conventional treatment for poor engraftment. The control group will receive only conventional treatment.

The primary outcome measures of the study include the rate of neutrophil engraftment, platelet engraftment, and red blood cell engraftment. Secondary outcome measures include the incidence of graft failure, the time to neutrophil and platelet engraftment, the incidence of acute graft-versus-host disease (GVHD), overall survival, and other relevant clinical outcomes.

The study will also assess the safety of UC-MSC treatment by monitoring adverse events, laboratory parameters, and immune-related parameters.

The research team plans to enroll a sufficient number of patients to achieve statistically significant results. Data analysis will be performed using appropriate statistical methods to compare the outcomes between the UC-MSC treatment group and the control group.

The study protocol has been developed in consultation with experts in the field and has received support from the National Key Research and Development Program Stem Cell Research and Organ Repair Major Project Subproject.

By investigating the use of UC-MSCs in the treatment of PGF after allo-HSCT, this study aims to contribute to the development of new treatment strategies for improving the engraftment of hematopoietic stem cells and enhancing the outcomes of allo-HSCT. The results of this study may have implications for the clinical management of patients undergoing allo-HSCT and provide insights into the potential therapeutic applications of UC-MSCs in hematopoietic stem cell transplantation.

Reference：

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2. Clinical Trial Objective

To explore the efficacy, safety, and related mechanisms of umbilical cord-derived mesenchymal stem cell therapy in the treatment of hematopoietic graft failure and engraftment dysfunction after allogeneic hematopoietic stem cell transplantation.

3. Clinical Trial Design

This is a single-center, randomized, controlled, open-label clinical trial designed to primarily investigate the efficacy and safety of umbilical cord-derived mesenchymal stem cell therapy in the treatment of engraftment dysfunction after allogeneic hematopoietic stem cell transplantation.

4. Study Population

4.1 Number of Cases

A total of 68 patients will be enrolled, with 34 patients in the experimental group and 34 patients in the control group.

4.2 Inclusion Criteria

The following criteria must be met for patient inclusion:

Patients undergoing allogeneic hematopoietic stem cell transplantation.
Both genders, aged ≥18 and ≤60.
Karnofsky Performance Status (KPS) score >60, estimated survival period >3 months.
No severe systemic impairment of vital organ function.
No contraindications to other hematopoietic stem cell transplantation.
Voluntary participation and informed consent.

4.3 Exclusion Criteria

Patients meeting any of the following criteria are not eligible for inclusion:
Severe heart, kidney, or liver dysfunction.
Patients requiring treatment for other malignancies.
Clinical symptoms of brain dysfunction or severe psychiatric disorders that affect comprehension or compliance with the study protocol.
Patients unable to complete the required treatment plans and follow-up observations.
Patients with severe acute allergic reactions.

Clinically uncontrolled active infections.
Patients currently participating in other clinical trials.
Participants deemed unsuitable for the clinical trial by the researchers.

4.4 Criteria for Removal or Dropout:

Patients experiencing disease progression unrelated to the experimental factors or death during the treatment and follow-up period will be unable to continue the observation.

4.5 Termination Criteria:

Occurrence of severe adverse reactions or intolerability.
Participant's voluntary withdrawal.
Disease progression or relapse during the trial period.
Participants deemed unsuitable for further treatment by the researchers.

5. Treatment Protocol

5.1 Treatment for Post-Transplantation Poor Graft Function (PGF) after Allogeneic Hematopoietic Stem Cell Transplantation

Experimental Group: UC-MSCs (umbilical cord-derived mesenchymal stem cells) for infusion will be provided by Chongqing Guolian Stem Cell Technology Co., Ltd., a subsidiary of Shanghai Saiao Biotechnology Co., Ltd. The umbilical cord tissue used as the source is obtained legally and meets ethical requirements. The UC-MSCs are not genetically modified or edited and undergo strict standard procedures and quality control measures during isolation, culture, testing, and transportation, ensuring their safety. The technologies "Clinical-Grade Umbilical Cord Mesenchymal Stem Cells and Method for Their Isolation and Preparation" and "Method for Isolation and Culture of Umbilical Cord Tissue-Derived Mesenchymal Stem Cells and Products Thereof" developed by Chongqing Guolian Stem Cell Technology Co., Ltd., a subsidiary of Shanghai Saiao Biotechnology Co., Ltd., have been granted patents by the National Intellectual Property Administration of China. The company has the qualification for UC-MSCs preparation, and the UC-MSCs prepared by them have been certified as qualified by a third-party testing institution. Therefore, UC-MSCs provided by Chongqing Guolian Stem Cell Technology Co., Ltd. will be used in this study. The preparation fee for UC-MSCs will be provided by the research funds of the National Key Research and Development Program "Stem Cell Research and Organ Regeneration," and the staff of the UC-MSCs preparation company will not participate in this study. In addition to routine PGF treatment, patients in the experimental group will receive umbilical cord blood-derived mesenchymal stem cells at a dose of 1×10^6/kg once a week for four consecutive weeks.

Control Group: Standard PGF treatment, including red blood cell and platelet transfusions, subcutaneous injection of granulocyte colony-stimulating factor (G-CSF) at a dose of 5-10ug/kg/day, subcutaneous injection of recombinant human thrombopoietin injection (TPO) at a dose of 300U/kg/day, and TPO receptor agonist

(TPO-RA) (starting dose of 1 tablet once daily, PLT<50×10^9/L for at least 2 weeks, increase by one dose level, PLT 200-400×10^9/L, decrease by one dose level, PLT>400×10^9/L, discontinue TPO-RA).

5.2 Observational IndicatorsI apologize, but the information you provided seems to be cut off.

6. Research Procedure

6.1 Recruitment and Baseline Evaluation

Subjects who meet the conditions of Section 4.1 of this research protocol will undergo a series of necessary functional examinations as essential research indicators for clinical studies. The main examination items include but are not limited to the following:

a. Physical examination (weight, height, surface area)

b. Vital signs examination: temperature, pulse, cardiac function

c. Complete blood count: hemoglobin, white blood cells, platelets, etc.

d. Liver and kidney function tests

e. Bone marrow examination, bone marrow biopsy

f. Minimal Residual Disease (MRD) and related gene mutation detection

g. Lymphocyte subset analysis

h. Imaging examinations: ultrasound, CT/MRI/DSA, etc.

6.2 Subject Document Collection

The documents required for subject recruitment include:

Completion of screening materials;

Signing the informed consent form;

6.3Implementation of Allogeneic Hematopoietic Stem Cell Transplantation

6.3.1 Preparative Regimen:

The preparative regimen for Haplo-HSCT patients is: Me-CCNU+Ara-C+Bu+Cy+ATG

Lomustine (Me-CCNU): 250mg/m2×1d

Cytarabine (Ara-C): 4g/m2×2d

Busulfan (Bu): 0.8mg/kg q6h, ×2d

Cyclophosphamide (CTX): 1.8g/m2×2d

Anti-thymocyte globulin (ATG): 2.5mg/kg×4d

The preparative regimen for SMD-HSCT patients is: Bu+Cy+ATG

Busulfan (Bu): 0.8mg/kg q6h×4d

Cyclophosphamide (CTX): 60mg/kg×2d

The preparative regimen for UMD-HSCT patients is: BU+CY+ATG
Busulfan (Bu): 0.8mg/kg q6h×4d
Cyclophosphamide (CTX): 60mg/kg×2d
Anti-thymocyte globulin (ATG): 2.5mg/kg×4d

6.3.2 Mobilization, Collection, and Infusion of Hematopoietic Stem Cells

Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is administered subcutaneously at a dose of 10ug/kg/day for 4 days. On day 5, peripheral blood stem cells (PBSC) are collected using the German Fresenius blood cell separator. On day 6, bone marrow aspiration is performed under epidural anesthesia. The donor lies prone on the operating table, and bone marrow is collected by puncturing at different depths and multiple sites on both iliac crests. If more than 600ml of bone marrow fluid is expected to be collected, the donor needs to be adjusted to a supine position, and bone marrow is collected by puncturing at the anterior superior iliac spine on both sides. If there is ABO blood type mismatch between the donor and recipient, hydroxyethyl starch sedimentation is used to remove red blood cells. If there is minor ABO blood type mismatch, the bone marrow fluid needs to be centrifuged at 4℃ and then plasma is removed using a plasma extractor.

6.4 Treatment of Post-Transplantation Graft Failure (PGF)

Experimental group: UC-MSCs (Umbilical Cord Mesenchymal Stem Cells) administered without any genetic modification or editing. In addition to routine treatment for PGF, umbilical cord mesenchymal stem cells are infused at a dose of 1×10^6/kg once a week for 4 consecutive weeks.

Control group: Conventional treatment for PGF, including red blood cell and platelet transfusion, subcutaneous injection of granulocyte colony-stimulating factor (G-CSF) at a dose of 5-10ug/kg/day, subcutaneous injection of recombinant human thrombopoietin injection (TPO) at a dose of 300U/kg/day, and TPO receptor agonist (TPO-RA) administration (starting dose of 1 tablet, once daily, for at least 2 weeks if PLT<50×10^9/L, increasing one dose level if PLT reaches 200-400×10^9/L, decreasing one dose level if PLT>400×10^9/L, discontinuing TPO-RA).

6.5 Observations of Indicators

Before hematopoietic reconstitution: Complete blood count (twice a week), liver and kidney function tests, coagulation function tests (weekly),cardiac function tests (as needed), and imaging examinations (as needed).

After hematopoietic reconstitution: Complete blood count (twice a week for the first month, then once a week for the second month, and once every two weeks for the third month), lymphocyte subset analysis (once a month for the first year), minimal residual disease (MRD) and related gene mutation detection (as needed), and imaging examinations (as needed).

Post-transplantation graft failure (PGF) observation: Complete blood count (daily), blood culture (as needed), liver and kidney function tests (as needed), coagulation function tests (as needed), and imaging examinations (as needed).

Long-term follow-up: Complete blood count (once every three months for the first year, then once every six months for the second year, and once a year thereafter), liver and kidney function tests (once every three months for the first year, then once every six months for the second year, and once a year thereafter), coagulation function tests (once every three months for the first year, then once every six months for the second year, and once a year thereafter), lymphocyte subset analysis (once a year), minimal residual disease (MRD) and related gene mutation detection (as needed), and imaging examinations (as needed).

6.6 Treatment Monitoring Plan

Pre-hematopoietic Reconstruction Monitoring Indicators:

(1) Changes in body temperature, pulse, and blood pressure (daily)

(2) Complete blood count (twice a week)

(3) Liver, kidney, and coagulation function tests (weekly)

(4) CMV, EB, and other viral indicators (1-2 times a week)

From hematopoietic reconstruction to 1 year post-transplant:

(1) Complete blood count, liver and kidney function tests, CMV and EB, and other viral indicators (at least monthly)

(2) Bone marrow examination, bone marrow biopsy (monthly)

(3) MRD (minimal residual disease), detection of relevant gene mutations (monthly)

(4) Lymphocyte subset analysis (1 month, 2 months, 3 months, 4.5 months, 6 months, 9 months, 12 months after transplantation)

6.7 Assessment Criteria for Treatment Efficacy

White blood cells: Neutrophil engraftment is defined as neutrophil count exceeding 0.5×10^9/L for three consecutive days.

Red blood cells: Hemoglobin level should be no lower than 70g/L, and the patient should no longer require blood transfusions.

Platelets:

Complete response (CR) is defined as a platelet count ⩾50×10^9/L for seven consecutive days without platelet transfusion.
Partial response (PR) is defined as a platelet count between 20×10^9/L and 50×10^9/L for seven consecutive days without platelet transfusion.
No response (NR) is defined as a platelet count <20×10^9/L or failure to discontinue platelet transfusion after eight weeks of treatment at the maximum tolerated dose.

6.8 Criteria for Assessing the Efficacy of Acute Leukemia Treatment

The evaluation of acute leukemia treatment is based on the 2017 edition of the "Chinese Clinical Diagnosis and Treatment Guidelines for Adult Acute Myeloid Leukemia (Non-Acute Promyelocytic Leukemia)" and the 2016 edition of the "Chinese Diagnosis and Treatment Guidelines for Adult Acute Lymphoblastic Leukemia." The assessment results may include the following possibilities: complete remission (morphological complete remission, molecular complete remission, morphological complete remission with incomplete blood cell recovery), relapse (molecular/genetic relapse, hematological relapse).

7. Safety and Adverse Events

7.1 Definition

Unexpected problems that may occur in patients or others: Any event, experience, or difficulty that meets the following conditions, including: unexpected nature, severity, or frequency (such as the absence of mentioned approvals from the Institutional Review Board (IRB) in study-related documents, protocols, informed consent forms, investigator manuals, etc.); related or potentially related to participation in the study (potential relevance means that there may be unforeseen events or results affecting the trial protocol in this study); indicating that the researcher puts patients or others at significant risk (including physical, psychological, economic, or social harm).

Adverse Event (AE): An adverse event is a symptom, sign, disease, or other development that reaches a severe level during the study process. Complications and injuries are considered adverse events. Abnormal diagnostic results that meet the following criteria are also considered adverse events: withdrawal from the study, association with many serious adverse events, association with clinical signs and symptoms, leading to additional treatment or further diagnostic tests, considered clinically significant by the study subjects.

Serious Adverse Event: Adverse events are categorized as serious or non-serious. Serious adverse events refer to the following adverse events: fatal, life-threatening,

requiring or prolonging hospitalization, causing persistent or severe disability, and other significant medical events.

Significant medical events refer to events that may not immediately threaten life but have significant clinical significance. It may jeopardize the patient or may require intervention to prevent the occurrence of other serious events mentioned above. For example, drug overdose or abuse, sudden onset of a disease that does not require hospitalization of the patient, intensification of bronchospasm treatment in the emergency department is usually considered serious. All other adverse events that do not meet the criteria for a serious adverse event are considered non-serious adverse events.

Dose-Limiting Toxicity (DLT): Dose-limiting toxicity is defined as Grade 3 or Grade 4 toxicity. Additionally, if the toxicity is autoimmune or natural allergy and occurs after repeated administration, Grade 2 toxicity is also considered DLT.

Adverse Event Reporting Period: The period during which adverse events occurring during the study need to be reported generally refers to the time from the start of the study to the end of the follow-up of study treatment. For this study, the follow-up of study treatment occurs within 6 months after the implementation of the last treatment. Adverse events occurring during chemotherapy and outcomes directly caused by antitumor chemotherapy are not within the adverse event reporting period.

Pre-Existing Condition: Pre-existing conditions refer to conditions that already exist at the start of the study. Pre-existing conditions need to be documented because frequent, severe, or worsening of pre-existing diseases may occur during the study process.

General Medical Findings: During the screening process, any clinically significant abnormalities are recorded as pre-existing conditions. After the study ends, any new clinically significant findings/abnormalities that meet the definition of adverse events must be recorded as adverse events.

Post-Study Adverse Events: All unresolved adverse events should be followed up by the investigator until they are resolved unless the patient is lost to follow-up or there is another explanation for the adverse event. At the last scheduled visit, the investigator needs to instruct each patient to report any events they or their personal physician believe may be related to their participation in the study. The investigator should note the occurrence of death events in the study or adverse events that occur

after some patients discontinue the study or terminate their participation in the study. The investigator should also note the possibility of cancer development in patients participating in the study or the presence of congenital abnormalities in offspring born to study participants.

Laboratory Abnormalities: If any of the following conditions occur in clinical laboratory abnormalities, they will be recorded as adverse events: abnormalities observed in the laboratory that have been proven to be abnormal through repeated testing and cannot be refuted in terms of their abnormality, abnormalities indicating the presence of diseases and/or organ toxicity. Abnormal levels need to be actively managed, such as dose adjustments, discontinuation of medication, more frequent follow-up assessments, further diagnostic investigations, etc. Laboratory abnormalities attributed to potential diseases and chemotherapy regimens are not considered adverse events (such as abnormal blood indices and mucositis).

Hospitalization, Prolonged Hospitalization, or Surgery: Any adverse event that leads to hospitalization or prolonged hospitalization should be recorded as a serious adverse event unless there are specific explanations in this protocol. Any condition responsible for surgery that meets the criteria for an adverse event needs to be recorded as an adverse event. As mentioned above, events related to antitumor chemotherapy are excluded from adverse events. Whether it is a condition, hospitalization, prolonged hospitalization, or surgery, it needs to be recorded as an adverse eventThe information provided above outlines the definitions and criteria for safety and adverse events in a clinical study. It includes the definition of adverse events, serious adverse events, dose-limiting toxicity, adverse event reporting period, pre-existing conditions, general medical findings, post-study adverse events, laboratory abnormalities, and hospitalization or surgery related to adverse events.

In summary, safety and adverse events are closely monitored during a clinical study to ensure the well-being of participants. Any unexpected problems, symptoms, diseases, or developments that occur during the study are recorded and evaluated. Adverse events are categorized as serious or non-serious, with serious adverse events encompassing severe or life-threatening events that may require hospitalization or cause significant medical consequences.

The reporting period for adverse events typically extends from the start of the study to the end of the follow-up of study treatment. Pre-existing conditions and any new clinically significant findings or abnormalities discovered during the study are documented as adverse events. Post-study adverse events, unresolved adverse events,

or events occurring after a participant's discontinuation of the study are also followed up and recorded.

Laboratory abnormalities indicating diseases, organ toxicity, or abnormalities that require active management are considered adverse events. Hospitalization, prolonged hospitalization, or surgery resulting from adverse events are also documented.

It's important to note that the information provided is a general overview, and specific protocols and procedures may vary depending on the clinical study and its objectives.

7.2 Adverse Event Recording

During communication with each patient, the investigator can collect information on adverse events through specific inquiries or, in certain specific situations, through testing. All information regarding adverse events needs to be immediately entered into the database and recorded in the Adverse Event module of the Case Report Form (CRF). All evident relevant signs, symptoms, and abnormal diagnostic results need to be entered into the database and grouped under the same diagnosis.

All adverse events occurring during the study period need to be recorded. All adverse events need to be tracked until resolution, stabilization, or confirmation that the study treatment or participation is not an issue. Serious adverse events that persist beyond the end of the study must be tracked until final resolution is determined. Any serious adverse events occurring after the study that are considered possibly related to the study treatment or participation need to be recorded and promptly reported.

7.3 Toxicity Management, Termination Rules, and Study Termination

7.3.1 Criteria for Study Termination or Suspension The study will be terminated if the following conditions occur:

If a subject experiences a Grade 3 or higher toxicity reaction following cell infusion therapy, identified as originating from the treatment protocol of this clinical trial, the clinical protocol will be modified to reduce the dosage of the treatment drug based on the nature of the toxicity reaction.

If the investigator or any independent review committee or regulatory authority determines that this trial would pose a safety risk to the subjects, the clinical trial will be terminated.

If the investigator decides to terminate this clinical trial, the trial will be terminated.

If any subject experiences the following conditions within 2 weeks after receiving cell infusion therapy, the study will be required to undergo reevaluation, and the clinical trial at that stage will be suspended:

Respiratory failure requiring mechanical ventilation.
Grade 4 (life-threatening) toxicity reaction resulting from the treatment protocol.
Death.

7.3.2Manegement of Toxic Effects

Infusion reactions: No literature reports of infusion reactions with MSCs have been observed. If they occur, anti-allergic and symptomatic treatments such as dexamethasone will be administered。

7.3.3 Criteria for Terminating Subject Participation in the Clinical Trial

If, after recruitment into the clinical trial, a subject cannot undergo allogeneic hematopoietic stem cell transplantation for various reasons, the subject will be terminated from the trial. This decision will be made by the principal investigator and may include factors such as changes in the underlying disease, severe infection, or significant organ dysfunction. Additionally, if relapse occurs during the MSC infusion period (within 12 weeks after transplantation), MSC preventive treatment will be terminated.

8.Sample Size Estimation

From the perspective of sample composition, the study is divided into the experimental group (MSC treatment group) and the control group. The estimation of sample size for one-sided testing using the two-sample rate comparison difference testing formula is calculated as c = n1/n2, with a statistically significant difference set at 0.05 and a detection power (P) value set at 0.80. The incidence rates in the experimental and control groups are 76% and 38%, respectively (based on previous historical control study results). Plugging these values into the formula yields a required sample size of 31 cases per group. Considering a possible dropout rate of 10%, each group will require 34 cases. The ratio of cases between the experimental and control groups is 1:1.

9.Data Processing and Record Keeping

9.1 Principle of Confidentiality

All information from the clinical trial is highly confidential. Except for inspections and duplication of records by regulatory authorities and sponsors or their representatives, no unauthorized personnel should be provided access to the information without written authorization from the sponsor. Unless authorized in

writing by the sponsor, all investigational drugs, subjects' biosamples, or other collected materials must be used according to the trial protocol.

In the case report form, the subjects' identities will be recorded using the uppercase initials of their names and unique subject codes.

9.2 Document Storage

To ensure evaluation and supervision by the National Medical Products Administration and the sponsor, the investigator should store all data from this clinical trial in accordance with relevant national regulations. This includes confirmation of all subjects (to effectively cross-check different records, such as CRFs and original hospital records and laboratory examination reports), all signed informed consent forms, all CRFs, and detailed records of drug distribution. The storage period is 5 years.

The sponsor should retain the trial protocol, ethics committee approvals, monitoring records, contracts, and records of serious adverse events for the required period as specified by the national drug regulatory authority.

Under no circumstances should the investigator provide any form of trial-related information to third parties.

9.3 Protocol Amendments

Once the trial protocol is determined and approved by the ethics committee, the research design should generally not be changed. However, modifications may be considered in the following two scenarios:

During the course of the trial, if it is found that it isnecessary to modify the protocol to ensure the safety and well-being of the subjects or to improve the scientific validity of the study, the investigator should submit a formal request for protocol amendment to the ethics committee for review and approval.

In exceptional circumstances, urgent modifications to the protocol may be required to eliminate immediate hazards to the subjects. In such cases, the investigator should immediately inform the ethics committee and obtain approval for the modification. The sponsor should also be notified promptly.

Protocol amendments should be documented and kept on file. All subsequent study procedures should adhere to the amended protocol, and the reasons for the amendments should be clearly stated in the final study report.

10. Statistics Analysis

Statistical analysis should be conducted by a qualified statistician using appropriate statistical methods. The analysis plan should be pre-specified in the protocol or a separate statistical analysis plan.

Descriptive statistics should be used to summarize baseline characteristics of the study population, including demographics, disease characteristics, and other relevant

variables.

For the primary outcome and secondary outcomes, appropriate statistical tests should be performed to compare the treatment groups. The choice of statistical tests should be justified based on the nature of the outcome variables and the study design. Confidence intervals and p-values should be reported for the estimated treatment effects.

For safety outcomes, adverse events should be summarized using appropriate descriptive statistics, including frequencies and percentages. Serious adverse events should be reported separately.

All statistical analyses should be conducted using appropriate statistical software, and the results should be reported in a clear and transparent manner.

11. Ethical Considerations

The study should be conducted in accordance with ethical principles outlined in the Declaration of Helsinki and applicable national and international regulations. The protocol should be reviewed and approved by an independent ethics committee or institutional review board before the study begins.

Informed consent should be obtained from all study participants or their legally authorized representatives. The informed consent process should provide clear and understandable information about the study, its risks and potential benefits, and the rights of the participants.

Privacy and confidentiality of the participants should be protected throughout the study. Personal information should be handled in accordance with applicable data protection laws.

The welfare and rights of the study participants should be prioritized. Any unexpected or serious adverse events should be promptly reported to the ethics committee, and appropriate actions should be taken to ensure the safety and well-being of the participants.